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Im Auftrag

For the President of the European Patent Office

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STARCH-BASED ENZYME GRANULATES

Field of the invention

The present invention relates to the formulation of enzymes, preferably feed-enzymes, into starch-containing granulates, and to processes for the preparation of such enzyme-containing granulates.

Background of the invention

The use of enzymes in animal feed for livestock has become almost common practice. The enzymes are produced by micro-organisms grown in large scale fermenters operated by industrial enzyme producers. At the end of the fermentation period, broths of micro-organisms are commonly subjected to a series of filtration steps to separate biomass from the enzyme. The enzyme solution is either sold as a liquid after addition of various stabilizers or processed to a dry formulation.

Enzyme liquids and dry formulations are used on commercial scale by the feed industry. Liquids may be applied to the feed after pelleting to prevent heat inactivation of the enzymes by the pelleting process. The problem is that the amounts of enzyme in the final feed preparations are very small which makes it difficult to realize a homogenous distribution of the enzyme molecules over the feed. Liquids are notoriously more difficult to mix than dry ingredients. One needs specific equipment to add liquids to the feed after pelleting which is not currently available at most feed mills.

Dry formulations of enzymes, on the other hand, have the disadvantage of heat-inactivation of the enzymes during pelleting. Preferred manufacturing conditions in the feed industry comprise steam pelleting, wherein the feed is

conditioned with steam injections prior to pelleting. In the subsequent pelleting step the feed is pressed through a matrix and the formed stripes are cut into suitable pellets of variable length. The moisture of the mass when arriving into the pelleting equipment is generally between about 18% and about 19%. During these process steps, temperatures may rise to 60-95°C. The combined effect of high moisture content and high temperatures is detrimental to most enzymes. These disadvantages equally apply to other types of thermomechanical treatments of feed, such as extrusion and expansion.

In order to overcome these problems EP 0 257 996 B1 discloses that the stability of enzymes in feed processing is increased by the preparation of an enzyme premix wherein an enzyme-containing solution is absorbed onto a carrier consisting of flour, which premix is subsequently pelleted and dried. Apparently this flour-based enzyme premix lends itself to pelleting. We have found, however, that such flour-based premixes are not at all suited for more gentle methods available for processing the dough-like premix into granulates, such as e.g. low-pressure extrusion or high shear granulation, because of the gluey character of the flour-based premixes.

Various enzyme manufacturers have developed alternative formulation methods to improve the stability of dry enzyme products during feed pelleting and storage. E.g. EP 0 569 468 B1 discloses that a formulation consisting of a enzyme-containing granulate that is coated with a high melting wax or fat would improve the resistance to pelleting conditions. In this instance the granulate is prepared by mixing a sodium sulphate carrier with the enzyme solution in a high shear granulator. EP 0 569 468 B1 teaches that the beneficial effect of the coating with respect to pelleting stability is specific for the type of granulate to be coated, which, in this case, is based on a sodium sulphate carrier. Unfortunately, however, the absorption capacity of the sodium sulphate carrier is much less than that of carriers such as flour, whereas there is a desire in the art to produce more concentrated enzyme-containing granulates.

There is thus a need in the industry for pelleting stable formulations of enzymes that are based on a carrier that is suitable for granulation methods other than pelleting and that has a high absorption capacity.

Description of the invention

The present invention provides processes for the preparation of enzyme formulations in the form of granulates that are based on the use of starch as a carrier. In these processes an enzyme-containing liquid, such as a solution or a slurry, is mixed with a solid carrier that is based on starch and is allowed to absorb onto the starch-based carrier. During or after the mixing, the mixture of the enzyme-containing liquid and the starch-based carrier is processed into a granulate, which is subsequently dried. The use of starch as solid carrier allows the absorption of large amounts of enzyme-containing liquid and further allows to form a plastic paste that can readily be processed into a pelleting stable granulate.

In the examples herein corn-, potato- and rice-starch is used. However, starch obtained from other sources such as tapioca starch, wheat starch, sago starch, rye starch, barley starch, sorghum starch, or arrowroot starch is equally applicable. Similarly both native or modified types of starch are applicable in the invention. Preferably the starch to be used in the invention contains little or no protein, i.e. less than 5% (w/w), more preferably less than 1% (w/w). Other suitable glucose-polymers that can be used in substitution for or in addition to starch include α-glucans, ß-glucans, pectines such as proto-pectin, glycogen and cellulose.

More than 15% (w/w) of the solid carrier consists of starch. Preferably, however, more than 30% (w/w) of the solid carrier consists of starch, more preferably more than 40% (w/w) of the carrier consists of starch. Most preferably the major part of the solid carrier consists of starch, i.e. more than 50% (w/w), preferably more than 60 % (w/w), more preferably

more than 70 % (w/w), and most preferably at least 80% (w/w) of the weight of the solid carrier consists of starch. The weight percentages referred to are based on the total weight of the non-enzymatic components in the final dry granulates.

The amount of enzyme-containing liquid that can be absorbed by the starch is limited by the amount of water that can be absorbed by the starch. For natural granular starch this amount varies between 25 - 30% (w/w) without using elevated temperatures that cause the starch to swell. Of course in practice the percentage of enzyme liquid to be added to the starch will be much larger than this 25 - 30% (w/w) because the enzyme liquids will usually contain a significant amount of solids. E.g. the enzyme solution used in the examples herein contain about 25% (w/w) solids as a result of which the starch and enzyme solution are mixed in a ratio of 60% (w/w) to 40% (w/w), respectively. Preferably the amount of enzyme liquid applied to the solid carrier is such that all the water in the liquid can be absorbed by the starch in the solid carrier. At higher temperatures starch can absorb much larger amounts of water under swelling. It is well known e.g. that corn starch can absorb about 300% water at 60°C and about 1000% water at 70°C, based on its original weight. The use of higher temperatures in order to absorb more enzyme-containing liquid is within the scope of the present invention, especially when dealing with thermostable enzymes. However, in general non-swelling conditions at lower temperature are preferred in view of activity losses due to instability of most enzymes at higher temperatures.

The mechanical processes that are applied in the present invention for processing the mixture of the enzyme-containing liquid and the starch-based carrier into granules, are frequently used in food, feed and enzyme formulation processes, where they occur in a number of ways such as expansion, extrusion, and spheronisation, pelleting, high shear granulation, drum granulation, fluid bed agglomeration or combinations thereof. All these processes are characterised by an input of mechanical energy, e.g the drive

of a screw, the rotation of a mixing mechanism, the pressure of a rolling mechanism of a pelleting apparatus, the movement of particles by a rotating bottom plate of a fluid bed agglomerator or the movement of the particles by a gas stream, or a combination thereof. These processes cause a powder, in this instance the starch-based solid carrier, to be mixed with an enzyme-containing solution or slurry, and subsequently form a granulate. Alternatively the solid carrier can be mixed with an enzyme powder to which a liquid or slurry is added, which acts as granulating liquid. In yet a further embodiment of the invention the granulate, or rather agglomerate, is formed by spraying the enzyme-containing liquid onto the starch-based carrier in a fluid bed agglomerator. For convenience sake we shall refer to a granulate (or granules) herein, also when granulate is in fact an agglomerate as produced in a fluid bed agglomerator. In any case the obtained granules can be further processed to give additional benefits, such as rounding off (spheronisation) or compaction.

Finally, the obtained granulates are dried in a (fluid bed) drier or, in case of the fluid bed agglomeration, can be immediately dried to obtain a solid dry granule. A number of well known methods for drying granulates in the food, feed or enzyme business are available to the skilled person.

In a preferred embodiment of the invention the mixing of the enzyme-containing liquid and the solid carrier additionally comprises kneading of the mixture with the aim to improve the plasticity of the mixture in order to facilitate granulation.

In case the granulate is formed by extrusion of the mixture of the enzyme-containing liquid and the starch-based solid carrier, the extrusion is preferably performed at low pressure. Low pressure extrusion offers the advantage that the temperature in the extruded mixture will not, or only slightly, increase. Low-pressure extrusion is herein understood to mean extrusion under conditions similar to

those occurring in e.g. in a Fuji Paudal basket- or dome-extruder.

In a preferred embodiment of the process according to the invention the obtained granules are spheronised prior to drying. Spheronisation will reduce dust formation in the final granulate and/or will facilitate coating of the granulate. Known methods for spheronisation are available to the skilled person including e.g. the use of a Marumerizer.

In a further preferred embodiment additional coatings are applied to the obtained granulates to give additional favoured characteristics or properties, like low dustiness, colour, protection of the enzyme from the surrounding environment, different enzyme activities in one product or a combination thereof. Preferably the granules are coated with a fat, a wax, a polymer, a salt, an unguent, a liquid containing a (second) enzyme or combinations thereof. It will be apparent that if desired several layers of (different) coating can be applied. For the application of the coating(s) onto the granules a number of state of the art methods are available which include the use of a fluid bed, a high shear granulator, a mixer granulator, or a Nauta-mixer.

In further embodiments according to the invention additional ingredients are incorporated into the granulate e.g. as processing aids, for further improvement of the pelleting stability and/or the storage stability of the granulate. A number of such preferred additions are provide herein below.

Salts may be added as additional constituent of the solid carrier. Alternatively however, in accordance with EP 0 758 018 A inorganic salts are added to improve the processing and storage stability of the dry enzyme preparation. More preferably inorganic salts comprising a divalent cation are used of which zinc, magnesium, and calcium salts are most preferred and sulphate as anion is most preferred. The salts may be added to the mixture in solid form. However, preferably, in accordance with EP 0 758 018 A, the salts are

dissolved in the enzyme-containing liquid prior to mixing with the solid carrier and drying of the granulate.

Further improvement of the pelleting stability is obtained by the incorporation of hydrophobic, gel-forming or slowly soluble compounds such as derivatised celluloses, such as HPMC (hydroxy-propyl-methyl-cellulose), CMC (carboxy-methyl-cellulose), HEC (hydroxy-ethyl-cellulose); polyvinyl alcohols (PVA); and/or edible oils. Edible oils, such as e.g. soy oil or canola oil, are added to the mixture to be granulated as processing aid. Surprisingly, we have found that the addition of such edible oils also increases the pelleting stability of the obtained enzyme granulate.

In a preferred embodiment according to the invention the granules have a narrow size distribution in order to facilitate a homogeneous distribution of the enzyme granulate in the animal feed. The processes according to the invention tend to produce granulates with a rather narrow size distribution. However, preferably an additional step is included in the process to further narrow the size distribution of the granulate, such as e.g. screening. The size distribution of the granulate is preferably between 100 μm and 2000 μm, more preferably between 200 μm and 1800 μm, most preferably between 400 μm and 1600 μm.

The enzyme-containing liquids to be used in the processes according to the invention comprise one or more enzymes and are usually obtained by microbial fermentation. Preferably the liquids are in a concentrated form, such as e.g. an ultra-filtrate, which will allow to produce a granulate with a desired activity level. Preferred enzymes to be included in the present invention are enzymes to be applied in animal feed. Animal feed is herein understood to also include pet food. The function of these enzymes is to improve the feed conversion rate, e.g. by reducing the viscosity or by reducing the antinutritional effect of certain feed compounds. Alternatively, feed enzymes (such as e.g. phytase) can be applied to reduce the amount of compounds which are

harmful to the environment in the manure. Preferred enzymes for these purposes are: phosphatases, of which preferably phytases and/or acid phosphatases; carbohydrases, such as amylolytic enzymes and plant cell wall degrading enzymes of which preferably cellulases such ß-glucanases, hemicellulases such as xylanases, or galactanases; proteases of which preferably proteases with a neutral and/or acidic pH optimum; and lipases of which preferably phospholipases such as the mammalian pancreatic phospholipases A2. It will be appreciated that in addition to enzymes the invention is equally applicable to non-enzymatic polypeptides with biological activities such as antigenic determinants to be used as vaccines and/or polypeptides engineered to have an increased content of essential amino acids, of which the biological activity may be sensitive to thermal inactivation.

The present invention further relates to enzyme-containing granulates that are produced in processes according to invention as described above. These granulates are suitable for use in processes for the preparation of an animal feed. In such processes the granulates according to the invention are mixed with feed substances, either as such, or as part of a premix. The characteristics of the granulates according to the invention allows their use as a component of a mixture which is well suited as an animal feed, if the mixture is steam treated and subsequently pelleted.

The following examples serve to illustrate the invention.

Examples

Materials and Methods

Extrusion tests were done using a Fuji Paudal DG-L1 basket extruder, with screen openings of 1.0 mm, screen thickness 1.2 mm, operating speed of 70 rpm, and an amperage of 0.6 - 2.0 A. The spheroniser was a Fuji Paudal Marumerizer QJ-400,

with a charge volume of 3 liters, plate pitch of 3 mm, retention time of 45-200 s and rotating speed of 750 rpm.

The high shear granulation tests were done using a Lödige type high shear granulator FM20, with a chopper speed of 1500 rpm and a ploughshare speed of 100 rpm. Powder was placed in this granulator an the enzyme-containing liquid was sprayed on top of this. The resulting granulates were dried in fluid bed drier

The enzyme solutions used were:

- an ultra-filtrate of an *Aspergillus* derived phytase with an activity of 16840 FTU/g, and a dry solids content of 22.4% (w/w).
- an ultra-filtrate containing a *Trichoderma* derived mixture of endo-xylanase and ß-glucanase activities of 12680 EXU/g and BGU/g, and a dry solids content of 20.6% (w/w).

Phytase activity was determined according to the procedure "ISL-method 61696" (manual vanadate assay).
ß-glucanase activity was determined according to the procedure "ISL-method 62170" (manual viscosimetric assay).
Endo-xylanase activity was determined according to the procedure "ISL-method 62169" (manual viscosimetric assay).
ISL-methods are obtainable on request from Gist-brocades, Food Specialties, Agri Ingredients Group, Wateringseweg 1, P.O. Box 1, 2600 MA, Delft, The Netherlands.

Example 1

Preparation of a corn starch-based enzyme granulate by kneading, extrusion, spheronisation and drying

An enzyme preparation was obtained by mixing and kneading a mixture of 60% (w/w) of corn starch with 40% (w/w) of an ultra-filtrate containing phytase. This mixture was extruded using the Fuji Paudal basket extruder to obtain a wet extrudate which was spheronised in the Marumeriser for one

minute to obtain round particles of an average diameter of 780 μm. These particles were subsequently dried in a fluid bed drier for 20 minutes at a bed temperature of 40°C, and an inlet temperature of 75°C. The thus obtained dry enzyme granules had an activity of 6980 FTU/g.

Example 2

Preparation of a corn starch-based enzyme granulate by high shear granulation and drying

The phytase ultra-filtrate and corn starch were mixed in a batch type high shear granulator of the Lödige type, with a batch size of 20 liters. The granulator was filled with 60% (w/w) of corn starch and 40% (w/w) ultra-filtrate was sprayed into the mixer during the mixing process. After addition of the ultra-filtrate (10 minutes) the granulator was mixed for another 5 minutes to allow the particles to be formed and compacted. The thus obtained granulate were dried in a fluid bed drier as mentioned in example 1. The resulting granules had an activity of 7420 FTU/g. The median diameter of the particles was 480 μm.

Example 3

Preparation of a corn starch-based enzyme granulate by mixing, pelleting and drying

A mixture of 40% (w/w) of the phytase ultra-filtrate and 60% (w/w) of corn starch was prepared. The mixture was pelleted using a Schlütter Press type PP85, where the extrudates were cut off by rotating knives at the extruder head, with a die plate containing holes of 1 mm in diameter. The pellets were dried as given in example 1, resulting in a final product with an activity of 7460 FTU/g. The median diameter of the particles was 1080 μm.

Example 4

Preparation of a potato starch-based enzyme granulate containing soy oil and $MgSO_4$ additions by mixing, kneading, pelleting and drying

In a mixer/kneader 30 kg of potato starch was added and 2.5 kg of Soy oil was mixed in. The soy oil acts as a processing aid and increases the pelleting stability of the obtained granules. Subsequently the phytase ultra-filtrate was added containing $MgSO_4.7H_2O$ (3.5 kg of $MgSO_4.7H_2O$ was dissolved in 14 kg of ultra-filtrate). The product was mixed thoroughly in the kneader and than extruded as mentioned in Example 1. The thus obtained granulate was dried in a fluid bed drier as mentioned in example 1. This resulted in a product of 5870 FTU/g.

Example 5

Preparation of a rice starch-based enzyme granulate by mixing, kneading, extrusion, spheronisation and drying

A mixture was prepared by mixing and kneading 62% (w/w) rice starch and 38% (w/w) of the phytase ultra-filtrate. This mixture was extruded using the Fuji Paudal basket extruder to obtain a wet extrudate which was spheronised in the Marumeriser for one minute to obtain round particles of an average diameter of 785 µm. These particles were subsequently dried in a fluid bed drier, as mentioned in example 1. The final activity of the granulate was 7280 FTU/g.

Example 6

Preparation of a corn starch-based enzyme granulate containing an HPMC addition by mixing, kneading, extrusion, spheronisation and drying

An enzyme preparation was obtained by kneading a mixture of 54% (w/w) of corn starch, 5% of HPMC (hydroxy-propyl-methyl-cellulose) and 41% (w/w) of an the phytase ultra-filtrate.

This mixture was extruded using the Fuji Paudal basket extruder to obtain a wet extrudate which was spheronised in the Marumeriser for one minute to obtain round particles of an average diameter of 780 µm. These were subsequently dried in a fluid bed drier for 20 minutes at 40°C bed temperature, and 75°C inlet temperature. The thus obtained dry enzyme granules had an activity of 8470 FTU/g.

Example 7

Preparation of a corn starch-based enzyme granulate containing an HEC addition by mixing, kneading, extrusion, spheronisation and drying

An enzyme preparations was obtained by mixing and kneading 54% (w/w) of corn starch, 5% (w/w) of HEC (hydroxy-ethyl-cellulose) with 41% (w/w) of the phytase ultra-filtrate. This mixture was extruded using the Fuji Paudal basket extruder to obtain a wet extrudate which was spheronised in the Marumeriser for one minute to obtain round particles of an average diameter of 780 µm. These were subsequently dried in a fluid bed drier for 20 minutes at 40°C bed temperature, and 75°C inlet temperature. The thus obtained dry enzyme granules had an activity of 6410 FTU/g.

Example 8

Preparation of a corn starch-based enzyme granulate by high shear granulation and drying

In a batch type high shear granulator of the Lödige type, with a batch size of 20 liters, 60% (w/w) of corn starch was mixed with 40% (w/w) of the ultra-filtrate containing endo-xylanase and ß-glucanase in the following manner: the granulator was filled with corn starch and the ultra-filtrate was sprayed into the mixer during the mixing process. After addition of the ultra-filtrate (10 minutes) the granulator was operated for another 5 minutes to allow the particles to be formed and compacted. The thus obtained granulates were

dried in a fluid bed drier as mentioned in example 1. The resulting granules had an activity of 13100 EXU/g and 5360 BGU/g.

Example 9

Comparison of the pelleting stabilities

The different enzyme granulates according to the invention were subsequently put in a pelleting trial and their pelleting stability was compared with those of the standard feed enzyme formulations. The pelleting trial consists of mixing the enzymes with a feed premix at 1000 ppm. This mixture was pretreated by injection of steam to give a temperature rise to 70°C, after which the mixture was pelleted in a pelleting machine to obtain the feed pellets, which are subsequently dried. This type of process is typical for the feed industry to obtain feed pellets.

Table 1 summarizes the results of the pelleting trials. It is apparent from Table 1 that the granule made using corn-, potato- or rice-starch provide improved pelleting yields as compared to the standard formulations. For NATUPHOS, the phytase containing formulation the standard is a mixture of wheat middlings with spray dried ultra-filtrate. For NATUGRAIN, the enzyme preparation containing ß-glucanase and endo-xylanase, this is a fluid bed granule, made by coating a salt core with an enzyme layer, which is applied to the core by spraying an ultra-filtrate onto this core.

Table 1. Results of the pelleting tests

	activity of the granules		Enzyme yield after pelleting at 70 C
	FTU/g	EXU/g BGU/g	%
Exp. 1	6980	-	54.9
Exp. 2	7420	-	51.8
Exp. 3	7460	-	62.8
Exp. 4	5870	-	62.7
Exp. 5	7280	-	54.7
Exp. 6	8470	-	69.6
Exp. 7	6410	-	67.3
Exp. 8	-	13100 5360	61.3 25.8
Standard NATUPHOS	5250	-	29.8
Standard NATUGRAIN	-	8150 6030	38.6 10.4

It is clear from Table 1 that the type of granulation methods, i.e. mechanical processing, does not really matter in the invention. Formulation using a starch provide a much better pelleting stability as compared to the current NATUPHOS and NATUGRAIN formulations. By adding components such as soy oil, HPMC or HEC the pelleting stability is increased even further.

CLAIMS

1. A process for the preparation of an enzyme-containing granulate wherein an enzyme-containing liquid and a solid carrier comprising at least 15% (w/w) starch are mechanically processed to obtain enzyme-containing granules, and wherein the enzyme-containing granules are subsequently dried.

2. A process according to claim 1, wherein said process comprises the steps of:

a) mixing an enzyme-containing liquid with a solid carrier comprising at least 15% (w/w) starch;
b) mechanically processing the mixture obtained in a) to obtain enzyme-containing granules;
c) drying the enzyme-containing granules obtained in b).

3. A process according to claim 1 or 2, wherein the mechanical processing is selected for the group consisting of extrusion, pelleting, high-shear granulation, expansion, fluid bed agglomeration, and combinations thereof.

4. A process according to any one of claims 1-3, wherein the mixing of the enzyme-containing liquid and the solid carrier additionally comprises kneading of the mixture.

5. A process according to any one of claims 1-4, wherein the extrusion is performed at low pressure, preferably in a basket- or dome- extruder.

6. A process according to any one of claims 1-5, wherein the granules obtained in step b) are spheronised, preferably in a Marumerizer, prior to drying in step c).

7. A process according to any one of claims 1-6, wherein the dried granules obtained in step c) are coated with a fat, a wax, a polymer, a salt, an unguent, or combinations thereof.

8. A process according to any one of claims 1-7, wherein a salt, preferably a salt comprising a divalent cation, is incorporated into the granulate.

9. A process according to any one of claims 1-8, wherein one or more hydrophobic, gel-forming or slowly soluble compounds are incorporated into the granulate.

10. A process according to claim 9, wherein the hydrophobic, gel-forming or slowly soluble compounds are selected from the group consisting of derivatised celluloses, such as hydroxy-propyl-methyl-cellulose, carboxy-methyl-cellulose, hydroxy-ethyl-cellulose; polyvinyl alcohols PVA; and edible oils, such as soy oil or canola oil.

11. A process according to any one of claims 1-10, wherein the enzyme-containing liquid comprises one or more enzymes selected from the group consisting of a phytase, an endo-xylanase and a ß-glucanase.

12. An enzyme-containing granulate characterized in that the granulate is produced in a process as defined in any one of claims 1-11.

13. A granulate comprising an enzyme and a solid carrier comprising more than 15% (w/w) starch as stabilizer.

14. A process for the preparation of an animal feed, characterized in that a granulate as defined in claims 12 or 13 is mixed with feed substances, either as such, or as part of a premix.

15. Use of granulate as defined in claims 12 or 13 as a component of a mixture which is well suited as an animal feed, if the mixture is steam treated and subsequently pelleted.

16. Use of a composition comprising more than 15% (w/w) starch as carrier for an enzyme, in a process of improving the pelleting stability of enzymes.

17. A process for promoting the growth of animals characterized in that the animal is fed a diet comprising the granulate as defined in claims 12 or 13.

ABSTRACT

A process for the preparation of an enzyme-containing granulate wherein an enzyme-containing liquid is mixed with a starch-based a solid carrier, mechanically processed into granules, and subsequently dried. The thus obtained starch-based enzyme granulate is suitable for use in the manufacture of animal feed compositions because of the improved enzyme stability during pelleting.